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EFFECTS OF INSPIRED OXYGEN ON THE METABOLISM OF PULMONARY SURFA--ETC(U)
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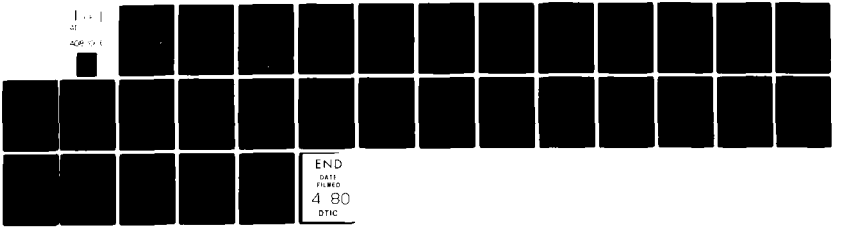

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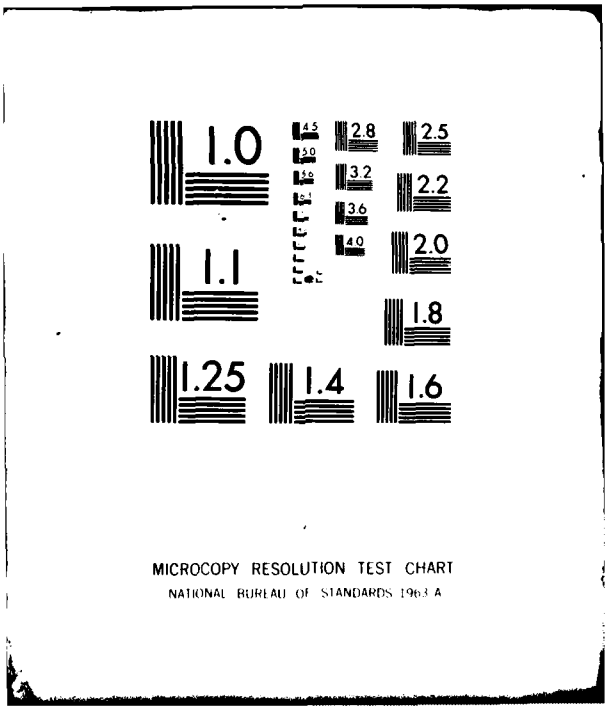
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Exposure to 100% O_2 at 1 atm. pressure has compromising effects on the ability of the lung to carry out gas exchange. The initial stages of injury include decreases in lung compliance, fluid accumulation in interstitial and alveolar spaces, and alveolar atelectasis. It is unknown whether pulmonary surfactant undergoes compositional or functional changes prior to or during the observed pathophysiology. Such alterations, if occurring, would interfere with normal physiological function and could exacerbate other effects of the environmental challenge.

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20. ABSTRACT (continued)

In order to better define alterations in surfactant that may result from breathing enriched concentrations of oxygen we exposed pathogen-free Wistar rats to 100% oxygen for 48 hours. We sacrificed the animals and separated the lipids in the alveolar wash fluid and in the lung homogenate, both known sources of surfactant pools. We used methods by which the major lipids constituting the intracellular and extracellular (alveolar) pools of surfactant could be quantified, paying particular attention to the content of disaturated phosphatidylcholine (DSPC). We found that the pool sizes of intracellular DSPC increased by 39% in animals exposed to 48 hours of 100% oxygen, changing from 21.5 mg DSPC per kg body weight (control) to 29.9 mg/kg (oxygen). Alveolar pools also increased by 82% going from 3.4 mg/kg (control) to 6.2 mg/kg (oxygen). Both changes were statistically significant ($P < 0.005$). We found no changes in the phospholipid composition of either pool of surfactant.

In separate experiments we injected ^{3}H-palmitate into rats and followed its incorporation and metabolism in the DSPC of Type II cells, alveolar surfactant, and alveolar macrophages. The results indicated that the DSPC moved from Type II cells to the alveolus, and at least part was ingested by alveolar macrophages. Overall metabolic flux was unchanged in animals exposed to oxygen.

We conclude that breathing 100% oxygen for 48 hours has direct effects on the amount of surfactant in the lung, but probably no effect on the metabolic pathways which regulate its phospholipid composition. These differences probably occur because of metabolic changes in catabolic processes associated with the clearance of surfactant. They may be indicative of early pathophysiological changes in lung cells which do not become demonstrable by morphology until longer exposures have occurred. Although obvious pathological effects are not evident after 48 hours exposure, 100% mortality occurred in 6 animals within an additional 24 hours of exposure.

EFFECTS OF INSPIRED OXYGEN ON THE METABOLISM OF PULMONARY SURFACTANT

I. Background

A. Physiological Effects of Breathing 100% Oxygen

It has been known since the turn of the century that breathing 100% oxygen can have deleterious effects on the respiratory system, and will eventually lead to death (1). The problem has assumed increased significance since 1940 as certain military weapon systems have required personnel to be subjected to environments containing above ambient concentrations of oxygen, and since oxygen is often required for the management of acute and chronic respiratory failure. The ultimate outcome of breathing 100% oxygen is without doubt (2). Structural damage to endothelial and Type I epithelial cells occurs, leading to a breach of the air-blood barrier. Fluid accumulates in interstitial and alveolar spaces, resulting in a deposition of fibrin debris products ("hyaline membranes"), an interference with the formation of function of pulmonary surfactant, alveolar atelectasis, and decreased diffusion of respiratory gases. The physiological consequences of this damage to lung tissue include the impairment of the uptake of oxygen, the development of pulmonary shunts, the mismatching of ventilation to perfusion, and the retention of carbon dioxide. Paradoxically, as these abnormalities develop, the organism becomes increasingly dependent upon respiration using hyperoxic breathing mixtures, resulting in a self-sustaining cycle of lung damage. The results that have been described are observed without exception. The principal variables that remain are: 1) the time over which this pathological condition develops; 2) the relationship between the severity of the pathological response and the concentration of oxygen being used; and 3) the biochemical basis for the cellular damage.

Pulmonary surfactant, the material which is synthesized by alveolar Type II cells and which lowers the surface tension of the alveolar interface (3), may be one of the substances in the lung which is perturbed by breathing above-ambient concentrations of oxygen. The surface properties of alveolar fluid are altered in some animals exposed to oxygen (4), but the mechanisms inducing these changes are unknown. A sustained insult to the lung by oxygen may result in a transudation of substances within the alveolar space (5). These may interact with the surfactant film, and prevent its manifesting normal physico-chemical properties (6). Alternatively, oxygen itself may affect the cells synthesizing and secreting this material, directly resulting in the inhibition of the metabolism of this material. The results regarding the direct effects of oxygen on the metabolic activities of Type II cells have been varied. Early work suggested that oxygen may perturb the metabolic activities of alveolar cells, but this viewpoint has been subsequently modified as the complexity of the pathophysiology of oxygen toxicity has

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become appreciated (2). Curiously, the question still remains unresolved, and takes an added significance because of recent studies on the cytokinetics of lung cells. Reports using both rodents (5) and primates (7) show that if an animal survives the initial damage induced by the elevated oxygen concentration, then it undergoes a pulmonary repair process which involves the proliferation of Type II-like cells. These cells re-occupy the alveolar epithelium, apparently in the attempt to repair the breached air-blood barrier. It becomes important, therefore, to determine if these cells are still capable of metabolizing surfactant in a normal manner.

Our experimental approach to this complicated pathophysiological problem is to concentrate upon studying the effects directly manifested by oxygen on the metabolic activities of the Type II cell. These experiments have required the development of new methodology: 1) techniques for isolating alveolar and intracellular pools of pulmonary surfactant and for quantifying the content of surfactant in these pools; and 2) methods for studying the metabolic turnover of pulmonary surfactant, specifically, in each of these respective pools. The results of the work supported in the first year of the grant have related to the first objective, the isolation and quantification of surfactant in alveolar and intracellular pools. The second year of the project has been devoted to the investigation of metabolic turnover rates. Alveolar pools have been recovered by endobronchial lavage of the lung. Intracellular pools have been studied through the isolation of alveolar epithelial Type II cells and alveolar macrophages. The content of dipalmitoyl phosphatidylcholine (DPPC), the principal constituent of surfactant (8), has been used to quantify this material, and its rate of metabolic turnover has been measured by isotopic techniques. The studies have been carried out on young (300 gm) specific pathogen free rats breathing room air (control animals) and in rats breathing 97% to 98% oxygen for 48 hours. This report describes the changes in the content and metabolism of DPPC in the lungs of animals breathing oxygen as compared with its content in normal animals, and speculates on the significance of these findings to the overall pathophysiology of oxygen poisoning.

II. Methods

A. Isolation of Alveolar Pools of Pulmonary Surfactant

Pathogen-free Wistar rats,* weighing about 300 gm, were obtained from Hilltop Laboratory Animals, Chatsworth, CA. and were used within 2 weeks of receipt as either control (air-breathing) or oxygen-exposed experiments. The animals were exposed to oxygen as described in Section II. D. Upon conclusion of the oxygen exposure the animal was anesthetized with sodium pentobarbital, weighed, and the lungs and intact trachea were exposed by midline incision. Alveolar surfactant was recovered from the lung by endobronchial lavage (9) using Hank's solution without calcium and magnesium (10). The lavages were combined and centrifuged at 1,500 rpm for 10 minutes to remove macrophages and other free cells. The supernatant fluid was centrifuged at 78,000 x g for 2 hours to pellet the surfactant. The pellet was suspended in 1 ml of water and placed in an ice bath for further procedures.

*The experiments reported herein were conducted according to the principles described in "Guide for Care and Use of Laboratory Animals, "DHEW Publication No. (NIH) 78-23, revised 1978.

In the interim the lung parenchyma was diced into small pieces and weighed. This tissue was used for the extraction and quantification of total intracellular lipid.

B. Analysis of Intracellular and Alveolar Lipids

Lipids were extracted from the lung tissue and the alveolar lavage lipids utilizing the method of Bligh and Dyer (11). The tissue was vigorously mixed in chloroform and methanol, using 1 ml chloroform and 2 ml methanol for each 0.8 gm of lung tissue. The mixture was homogenized for 5 minutes using a Tekmar power homogenizer (Tekmar, Cincinnati, Ohio) until the tissue was dispersed into very fine pieces. One additional ml of chloroform was added and the mixture was again homogenized for 2 minutes. The extract was filtered on a Buchner funnel and the tissue was scraped off the filter paper. This was mixed in the beaker with an additional ml of chloroform and homogenized a third time for 2 minutes. The additional extract was filtered and the filter and beaker was washed with 0.5 ml of fresh chloroform. All filtrates were combined and an internal standard of dipalmitoyl phosphatidylcholine labeled with ^{14}C in the 1 carbon position (New England Nuclear) was added to the filtrate. One ml of water was added, the mixture was mixed vigorously for 2 minutes and centrifuged for 5 minutes to obtain a separation between the water-enriched upper phase and the chloroform-rich lower phase. The top layer was removed and the lipid in the bottom layer was washed with an additional ml of water. The phases were again separated by centrifugation and the top layer was removed. The bottom layer was evaporated under a stream of nitrogen at 40-45°C, and the residue was dissolved in 1 ml chloroform for further lipid analysis. The lipids in the pellet of alveolar surfactant were extracted in a similar manner. Total lipids were quantified by dry weight and by phosphorus content (12).

Neutral lipids were separated from the phospholipids on a column of silicic acid. For each mg of total lipid 100 mg of silicic acid were activated for 1 hour at 110°C. The silicic acid was mixed with chloroform to make a smooth slurry and poured into a small glass column. The packed column was washed with 5 column volumes of chloroform, and the total lipids in chloroform were applied to the column. The column was washed with chloroform (10 ml for each gm of silicic acid) to elute the neutral lipids. The chloroform elution was followed with 5:4 (V/V) chloroform/methanol (10 ml per gm) to remove most of the phospholipids. To ensure total recovery of phospholipid an additional wash of methanol (15 ml per gm of silicic acid) was used to further strip the column. The chloroform/methanol and total methanol eluates were combined. All fractions were evaporated in a rotary vacuum evaporator at 40°C. The residues were dissolved in 1 ml chloroform and aliquots were taken for determination of phosphorus and dry weight.

The individual phospholipids were separated using silicic acid thin layer chromatography. A 250 μ silica gel G-plate (Analabs) was activated for 1 hour at 110°C. Samples of the mixed phospholipids were spotted 2 cm from the bottom of the plate and were allowed to migrate 16

cm into the plate using a solvent system of 60:35:5 (V/V) chloroform/methanol/water. The plate was dried in air and the spots were visualized with iodine vapor. Phospholipids were identified by comparison with the migration of known standards. The lipids were carefully scraped into phospholipid assay tubes and quantified by phosphorus content. This procedure was capable of separating and quantifying lysophosphatidylcholine, sphingomyelin, total phosphatidylcholines and phosphatidylethanolamine/phosphatidylglycerol (migrating together).

To further resolve the total phosphatidylcholines into fully saturated and unsaturated phosphatidylcholines, and to separate the phosphatidylglycerol from the phosphatidylethanolamine, additional steps of chromatography were required. On separate silica gel G thin layer plates the total phosphatidylcholines and the phosphatidylglycerol/phosphatidylethanolamine fractions are separated from the other phospholipids. These phospholipids were scraped from the silica gel plate into test tubes and eluted from the gel with 97:97:6 (V/V) chloroform/methanol/water. The elution was accomplished by mixing the phospholipid vigorously with the solvent, centrifuging to separate the gel and removing the supernatant. The procedure was repeated 3 times. The eluates were evaporated under nitrogen at 40-45°C utilizing small additions of methanol to assist in the removal of the water. The phospholipid residues were dissolved in 1 ml of chloroform for further analysis. Samples were taken for dry weight, phosphorus and of ^{14}C radioactivity to characterize the recovery of ^{14}C-dipalmitoyl phosphatidylcholine.

The material migrating with the same Rf as the phosphatidylethanolamine standard was principally comprised of phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). These were separated using a 250 μ silica gel G plate saturated with an 0.8 molar solution of boric acid. The plate was dried for at least 1 hour at room temperature and activated for 2 hours at 110°C. The entire PE/PG fraction was applied to the plate and the plate was developed with 70:30:3:2 (chloroform:methanol:water:concentrated ammonia). The plate was dried in air at room temperature and the spots were visualized by iodine vapor. The spots were individually removed for quantification by phosphorus and dry weight, and for gas-liquid chromatography of the esterified fatty acid.

The total phosphatidylcholine fraction was separated into fully saturated phosphatidylcholines and unsaturated phosphatidylcholines utilizing a modification of the method developed by Mason and coworkers (13). The mixed phosphatidylcholines were evaporated to dryness under nitrogen at room temperature. Osmium tetroxide dissolved in carbontetrachloride at a concentration of 1 mg/100 ml was added to each sample using 1 mg of osmium tetroxide for each mg of phospholipid. The solution was mixed well and allowed to react at room temperature for 1.5 hours. Upon completion of the reaction the sample was evaporated at room temperature under a stream of nitrogen and dissolved in 0.5 ml of chloroform/methanol/ammonia (50:49:1). It was then applied to a column of silicic acid equilibrated with the same solvent mixture, using 150 mg of silicic acid for each 500 μg of phospholipid. The column was eluted

with 10 ml of chloroform/methanol/ammonia (50:49:1). Most of the fully saturated phosphatidylcholines were eluted from the column with the solvent while the reaction product of the unsaturated phosphatidylcholines was adsorbed to the column. The eluates were dried under nitrogen at room temperature and dissolved in a known volume of chloroform. Aliquots were taken for phosphorus, ^{14}C-radioactivity, and gas-liquid chromatography. Lipids were quantified by content of phosphorus, and by the recovery of the internal standard of ^{14}C-labeled dipalmitoyl phosphatidylcholine. Gas-liquid chromatography was used to verify the content of saturated fatty acids in the phosphatidylcholine fraction.

The analysis of fatty acid residues was carried out by standard gas-liquid chromatography procedures (14). Hydrolysis and esterification of the phosphatidylcholine was carried out in 1% H_2SO_4 in methanol heated for one hour at 70°C. The samples were cooled to room temperature, mixed with 1 ml of water and 2 ml of hexane and centrifuged in a table-top centrifuge at room temperature. The fatty acid methyl esters were recovered in the hexane phase and were evaporated to dryness at room temperature under nitrogen, dissolved in 5 to 10 µl of carbon disulfide, and injected into a gas-liquid chromatograph. Chromatography was carried out in a Hewlett-Packard Model 5830A GLC using a 6 ft. column of 10% SP 2340 on 100/120 mesh Supelcoport (Supelco, Belleforte, PA.) run isothermally at 175°C. Quantification was carried out by computer integration of the area under the curve.

C. Measurement of Metabolic Turnover

Rats of both sexes weighing about 300 gm were exposed to either air or 100% oxygen for 48 hours, as described below. The animals were removed from the chamber and anesthesized lightly with sodium pentobarbital (8 mg/kg body weight) administered i.p. The jugular vein was isolated through a small incision in the neck, and was cannulated for injection of ^{3}H-palmitic acid (9,10-^{3}H obtained from New England Nuclear, specific activity 10-30 Ci/mmole). The palmitic acid was injected as a buffered aqueous solution complexed to albumin. We used 0.75 mCi/kg isotope for control animals and 1.0 mCi/kg for animals exposed to oxygen. The incision was sutured and the animals were allowed to recover from anesthesia. At times varying from 15 minutes to 16 hours the animals were re-anesthesized with sodium pentobarbital, the chest was opened, and the lungs were perfused with Minimum Essential Medium through the pulmonary artery. Alveolar epithelial Type II cells, alveolar surfactant, and alveolar macrophages were obtained by methods described previously (22). Populations of cells which were enriched in Type II cells contained 74.6% (S.D. 9.3) Type II cells in control animals and 64.1% (S.D. 12.7) in oxygen-exposed animals. Alveolar macrophage populations were greater than 95% pure in controls, and greater than 90% pure in oxygen animals. The major contaminating cells in the latter animals were polymorphonuclear leukocytes and lymphocytes.

The lipids in the purified fractions were extracted with chloroform/methanol (11), and the DSPC was isolated by silicic acid chromatography after treatment of the lipids with osmium tetroxide (13). The specific

activity of the ^{3}H-palmitate incorporated into the DSPC was measured by quantitative GLC using an external standard of dinonadecanoyl-L-α-phosphatidylcholine (Supelco, Inc., Bellefonte, Pa.), together with scintillation counting. Most of the methodology has been described in previous papers (22,23).

D. Exposure of Animals to Oxygen

Pathogen-free rats were exposed in a plexiglass chamber (24 in. x 24 in. x 24 in.) using 100% oxygen in an open flow-through system. Oxygen flows of 10-15 liters/min. were used to maintain oxygen concentrations within the chamber at a constant 97% to 98%. Carbon dioxide levels were less than 0.06% and temperature and humidity were about those of ambient levels. Oxygen concentrations of the effluent gas in the chamber were continuously monitored by Beckman oxygen meter and were stable throughout the 48 hr. period of exposure. At the completion of the exposure the animals were removed from the chamber and allowed to breathe air for 30 min. During this period of air breathing the animals were anesthetized and prepared for surgery.

III. Results

A. Separation of Lipid Constituents

Column and thin-layer chromatography using silicic acid was capable of separating total neutral lipid from phospholipid, as well as resolving lysophosphatidylcholine, sphingomyelin, total phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and the percentage of disaturated phosphatidylcholine in the total phosphatidylcholine fraction. Disaturated phosphatidylcholine was well separated from the total phosphatidylcholine fraction after treating with osmium tetroxide. Gas-liquid chromatography of the disaturated phosphatidylcholine indicated that its average fatty acid saturation was 97% for the intracellular pool and 98% for the alveolar pools. Greater than 90% of the ^{14}C-dipalmitoyl phosphatidylcholine in the total phosphatidylcholines was recovered with the saturated phosphatidylcholines.

B. Quantification of Lipids

After 48 hrs. of exposure to 97% oxygen we found that both the intracellular and alveolar pools of disaturated phosphatidylcholine increased. The data are shown in Tables 1 and 2 and on Figures 1 and 2. In Tables 1 and 2 disaturated phosphatidylcholine is expressed as total disaturated phosphatidylcholine recovered per lung, as disaturated phosphatidylcholine per kg of body weight, and as disaturated P.C. per gram lung weight. Alveolar disaturated phosphatidylcholine increased by 82% after exposure to oxygen going from 3.4 mg/kg (control) to 6.2 mg/kg (oxygen). The total amount of intracellular disaturated phosphatidylcholine increased by 39% in animals exposed to oxygen, changing from 21.5 mg/kg (control) to 29.9 mg/kg (oxygen). Both changes were statistically significant, $P < 0.005$. Similar changes would be reflected in the data expressed as total disaturated phosphatidylcholine recovered from each of these compartments. The changes were less impressive when

expressed as mg per gram lung weight, as lung weight increased in animals exposed to oxygen. In two animals we quantified the changes in pool size after exposure of the animals to 48 hrs. of 97% oxygen, followed by a 24 hr. "recovery period" breathing room air. Intracellular pools of phosphatidylcholine were about the same as those found for animals breathing 48 hrs. oxygen, while alveolar pools were increased to an even greater extent. The data are shown as 2 data points on Figures 1 and 2.

We investigated whether the changes in pool size were also associated with changes in the composition of the intracellular and alveolar pools. Analyses of phospholipid and neutral lipid compositions as well as their fatty acid moieties did not reveal any significant changes in composition in the lung lipids, or in the alveolar pool lipids. The data are presented in detail in Tables 3-12. Total intracellular lipids were comprised of about 70% phospholipid and 30% neutral lipids, and this ratio was unchanged after exposure to oxygen (Table 3). Saturated phosphatidylcholines comprised about 30% of the total phospholipid, with the remainder comprised of 12% sphingomyelin, 20% unsaturated phosphatidylcholine, 20% phosphatidylethanolamine, 8 to 10% lysophosphatidylcholine and small amounts of phosphatidylglycerol (Table 5). The fatty acid composition of the total phosphatidylcholines, phosphatidylethanolamines and phosphatidylglycerols are shown in Tables 7, 9 and 11. There were no detectable changes with exposure to oxygen.

Alveolar lipids were comprised of about 80% phospholipid and 20% neutral lipid (Table 4). Saturated phosphatidylcholines comprised about 60% of the total phospholipid and the remainder of the phospholipid was made up of about 10% unsaturated phosphatidylcholine, 3-4% phosphatidylethanolamine, 13% phosphatidylglycerol and small amounts of sphingomyelin and lysophosphatidylcholine (Table 6).

The fatty acid compositions of the phosphatidylcholines and the phosphatidylglycerols showed a relatively high percentage of saturated fatty acid residues, as shown in Tables 8 and 10. The phosphatidylethanolamines were less saturated than those of the other phospholipids, and were comprised of about 60% saturated fatty acids and 40% unsaturated fatty acids. Phosphatidylethanolamines were the only phospholipids in the alveolar lipid pool which contained substantial amounts of arachidonic acid (20:4). There were no statistically significant differences in the distribution of phospholipids or in their fatty acid compositions after exposure of the animals to 48 hours of oxygen.

C. Metabolic Turnover

The data describing the changes in specific activity of the DSPC in alveolar cells and in alveolar surfactant are given in Figures 3 and 4. The specific activity of the DSPC in Type II cells obtained from control animals is maximal by the time of sacrifice at 15 minutes, and declines monotonically with time. Maximum labeling of alveolar surfactant occurs at 6 hours, while that in alveolar macrophages is not yet evident by the time of the 16-hour sample. The pattern of labeling suggests a transfer of the DSPC from Type II cells to the alveolar lumen to alveolar macrophages, and is similar in both control and oxygen-exposed animals.

The change in specific activities of the DSPC in Type II cells from control and oxygen animals is compared in Figure 5. The data for both groups of animals fit a first-order equation reasonably well (r=0.85, control; r=0.74, oxygen), and are plotted on semi-logarithm paper. We can estimate the half-life of the first-order process from the slopes of the curves if we assume that the immediate precursor pools are pulse-labeled, and that the kinetics represent the metabolism of one intracellular pool of DSPC. The calculation indicates a half-life of DSPC in control cells of 3.2 hours and that in cells from oxygen-exposed animals of 4.8 hours. An analysis of covariance indicates that the regressions are not statistically different ($P > 0.10$).

D. Mortality

All animals used in this study survived exposure to 97% oxygen for 48 hours. We exposed 6 rats to an additional 24 hours of oxygen, and all died within the 72-hour time period.

IV. Discussion

The results indicate that the amounts of disaturated phosphatidylcholines which can be recovered from the alveolar space or from intracellular sites are increased in response to breathing 100% oxygen for 48 hours. These findings are similar to data reported by Valimaki, et al (15), who studied rats exposed to 100% oxygen for 60 hours. They would seem, at first perusal, to be in contrast with the expected effects of breathing 100% oxygen which generally results in changes in enzyme patterns which inhibit metabolic activities (16). The differences may reflect a unique metabolic adaptation by the alveolar epithelial Type II cell to an environment of high P_{O_2}. Observations that have been made on the lung as it responds to a variety of insults, including high oxygen (7), nitrogen dioxide (17), ozone (18), inhaled chemicals (19) and bacteria indicate that the Type II cell has a relativelyhigh survival in these challenging environments. Our recent observations bear this out. The pools of both intracellular and extracellular DSPC are elevated, and this may be indicative of the unimpaired synthesis and secretion of pulmonary surfactant. The half-life of the intracellular metabolism of the DSPC of oxygen-exposed animals is decreased by about 32%, but it is not statistically different from that of control animals. Total metabolic flux is a product of turnover rate times pool size. Even though turnover rate is decreased by 32%, intracellular pool size is elevated by 39%. (We assume that the change in the pool of DSPC in Type II cells isproportional to the change in the pool in DSPC of whole tissue). Thus, the overall metabolic flux of surfactant DSPC after exposure to oxygen is probably unchanged. The reason for the increase in the pools of DSPC is unknown. Our results do not indicate that it has resulted from primary perturbations to synthetic machinery. We think that the most logical assumption, therefore, is that exposure to oxygen has resulted in changes in the mechanism by which surfactant is removed from the alveolar space. Several reports now indicate that alveolar macrophages participate in the clearance process (21,22).

Conceivably the breathing of 100% oxygen might inhibit macrophage function, and this inhibition may become detectable after 48 hours exposure. Although possible, it would represent a pathophysiological condition whose importance may not be manifest until longer terms of exposure are encountered. Other pulmonary damage, including the destruction of Type I epithelial and endothelial cells, would also occur during these longer exposures, and its consequences may be more debilitating than that expressed through defects in the catabolism of surfactant.

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Table 1. Content of DPPC in Lung Tissue

	Control			97% Oxygen		
	Amount (mg)	Amount Body Wt. (mg/Kg)	Amount Lung Wt. (mg/g)	Amount (mg)	Amount Body Wt. (mg/Kg)	Amount Lung Wt. (mg/g)
	6.2	21.5	5.9	9.1	30.4	6.0
	7.7	25.9	7.6	10.2	30.7	6.7
	6.9	20.2	5.3	7.2	25.9	6.8
	6.1	20.6	4.9	8.3	31.4	7.8
	6.1	19.5	5.6	8.6	27.2	5.6
				10.0	33.7	6.5
Mean	6.6	21.5	5.9	8.0	29.9	6.3
S.D.	0.7	2.5	1.0	3.2	2.4	0.8

Table 2. Content of DPPC in Alveolar Fluid

	Control			97% Oxygen		
	Amount (mg)	Amount / Body Wt. (mg/Kg)	Amount / Lung Wt. (mg/g)	Amount (mg)	Amount / Body Wt. (mg/Kg)	Amount / Lung Wt. (mg/g)
	1.0	3.0	0.8	2.2	7.2	1.4
	1.0	3.1	N.D.	2.8	8.5	1.8
	1.0	3.5	N.D.	1.3	4.7	1.2
	0.9	3.3	N.D.	1.3	4.9	1.2
	1.3	4.4	1.1	1.8	5.6	1.2
	1.0	3.2	0.9	1.9	6.5	0.9
Mean	1.1	3.4	0.9	1.9	6.2	1.3
S.D.	0.1	0.5	0.1	0.6	1.5	0.3

Table 3. Distribution of Lipids Between Neutral and Phospholipids in Lung Tissue

	Control		97% Oxygen	
	Phospholipids (% of Total)	Neutral	Phospholipids (% of Total)	Neutral
	69.8	30.2	58.4	41.6
	71.7	28.3	61.7	38.3
	57.7	42.3	64.1	35.9
	67.6	32.4	74.4	25.6
	63.8	36.2	58.1	41.9
			64.9	35.1
Mean	66.1	33.9	63.6	36.4
S.D.	5.5	5.5	6.0	6.0

Table 4. Distribution of Lipids Between Neutral and Phospholipids in Alveolar Fluid

	Control		97% Oxygen	
	Phospholipids (% of Total)	Neutral	Phospholipids (% of Total)	Neutral
	61.0	39.0	78.4	21.6
	76.2	23.8	94.6	5.4
	76.1	23.9	85.0	15.0
	71.6	28.4	82.1	17.9
	86.0	14.0	74.7	25.3
	70.0	30.0	85.1	14.9
Mean	73.5	26.5	83.3	16.7
S.D.	8.3	8.3	6.8	6.8

Table 5. Distribution of Phospholipids in Lung Tissue

	Lyso-P.C.	Sphingomyelin	Saturated P.C.	Unsaturated P.C.	P.E.	P.G.
		(% of Total Phospholipid)				
Control						
		9.3	36.8	15.6	29.8	6.2
		13.9	33.1	25.4	20.9	3.7
		9.2	29.7	30.5	24.5	3.4
	9.3	15.2	27.7	20.2	23.6	2.2
	9.4	14.8	29.1	19.9	22.2	2.4
Mean	3.7	12.5	31.3	22.3	24.2	3.6
S.D.	5.1	3.0	3.7	5.7	3.4	1.6
97% Oxygen						
	8.0	12.8	31.1	19.8	21.6	5.8
	9.3	11.8	29.5	19.8	22.6	6.1
	9.1	15.0	27.2	19.0	25.3	3.7
	8.9	12.6	30.0	20.0	24.8	3.1
	6.6	12.1	29.5	22.2	15.1	3.5
	6.7	11.4	31.9	21.2	15.4	2.5
Mean	8.1	12.6	29.9	20.3	20.8	4.1
S.D.	1.2	1.3	1.6	1.2	4.5	1.5

Table 6. Distribution of Phospholipids in Alveolar Fluid

	Lyso-P.C.	Sphingomyelin	Saturated P.C.	Unsaturated P.C.	P.E.	P.G.
Control						
	4.9	1.1	54.3	23.4	2.7	13.6
	14.0	4.6	55.5	9.2	2.4	14.3
	9.4	5.4	58.9	8.1	4.4	13.9
	11.6	5.3	54.7	12.7	4.4	11.3
	4.3	1.6	70.1	2.9	2.2	12.1
	4.7	1.9	62.3	9.0	3.2	12.6
Mean	8.2	3.3	59.3	10.9	3.4	13.0
S.D.	4.1	2.0	6.1	6.9	0.8	1.2
97% Oxygen						
	7.0	4.2	67.6	7.4	3.3	8.6
	8.4	1.7	72.8	3.7	1.6	10.2
	4.4	1.4	60.4	20.2	1.6	10.7
	6.9	2.3	55.2	21.8	1.5	11.0
	4.6	2.5	58.8	14.9	0.4	12.6
	6.3	3.5	57.2	16.1	0.3	11.9
Mean	6.3	2.6	62.0	10.5	1.5	10.8
S.D.	1.5	1.1	6.8	7.7	1.1	1.4

Table 7. Distribution of Fatty Acid Residues in Total Phosphatidylcholines in Lung Tissue

14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Control							
2.7	48.0	6.3	9.8	12.1	8.2	—	7.4
2.8	50.2	4.3	10.8	12.5	8.1	—	11.4
2.6	50.3	7.0	10.0	11.6	7.3	—	8.1
2.2	50.9	6.6	9.0	11.5	9.4	—	7.6
2.1	49.6	6.5	9.6	11.5	8.7	—	9.0
97% Oxygen							
2.5	50.3	7.0	9.2	9.4	8.0	—	10.0
1.9	50.5	7.3	9.1	10.4	7.4	—	8.8
1.6	47.7	7.2	10.3	11.8	8.3	—	10.4
1.6	47.7	7.2	10.2	10.3	9.2	—	10.4
2.3	51.5	7.9	8.5	10.6	7.9	—	8.1
1.9	50.6	8.2	9.0	10.4	7.9	—	9.0

Table 8. Distribution of Fatty Acid Residues in Total Phosphatidylcholines in Alveolar Fluid

14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Control							
4.5	72.1	8.9	3.5	3.9	4.0	—	2.5
7.0	73.5	8.1	3.1	2.0	3.0	—	1.2
6.5	70.8	8.0	4.3	2.4	4.0	—	3.3
6.1	77.5	7.6	3.4	2.0	2.1	—	0.7
5.1	74.9	8.7	3.1	2.5	3.4	—	1.5
4.7	74.6	8.0	3.1	3.3	3.7	—	1.7
97% Oxygen							
4.9	74.3	7.9	3.7	2.4	3.0	—	1.7
3.6	73.3	9.4	3.6	3.4	3.4	—	1.5
3.0	71.2	11.0	3.0	3.7	4.7	—	2.2
3.6	69.8	11.4	3.8	3.4	4.9	—	2.1
4.8	73.0	10.4	3.0	2.8	3.4	—	1.7
3.2	71.9	10.8	3.7	3.0	4.2	—	1.7

Table 9. Distribution of Fatty Acid Residues in Phosphatidylglycerol in Lung Tissue

14:0	16:0	16:1	18:0	18:1	18:2	18:3	*20:4
Control							
3.3	20.9	—	8.7	46.7	8.4	—	2.1
—	35.3	—	2.1	19.7	3.6	—	39.3
1.2	29.2	1.5	2.0	14.2	3.1	—	39.0
1.0	55.6	4.9	6.0	14.8	10.5	—	1.4
1.4	58.0	—	8.8	15.9	12.1	—	3.5
97% Oxygen							
—	21.9	2.1	5.4	17.5	8.7	—	35.3
0.4	33.0	4.2	6.1	21.6	8.7	—	23.6
1.2	42.5	4.4	2.9	18.6	4.7	—	21.6
1.5	56.0	6.8	4.0	17.1	7.0	—	7.5
0.3	46.3	8.2	9.1	17.1	14.1	—	4.9
0.7	52.6	6.9	8.3	16.4	11.7	—	2.9

* Varying amounts of 20:4 probably result from a contaminant of the phosphatidylglycerol fraction which could not be resolved in all experiments.

Table 10. Distribution of Fatty Acid Residues in Phosphatidylglycerol in Alveolar Fluid

14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Control							
1.8	67.1	*N.D.	5.5	12.7	8.2	—	4.4
2.2	73.8	N.D.	4.3	11.3	5.9	—	2.5
1.7	66.6	N.D.	6.1	14.7	6.7	—	4.1
1.7	76.2	N.D.	4.3	12.1	4.1	—	1.7
2.0	71.8	N.D.	4.8	10.8	8.1	—	1.8
2.3	71.9	N.D.	5.1	11.0	7.8	—	1.5
97% Oxygen							
1.8	70.7	—	5.7	11.6	9.7	—	—
1.3	61.8	3.9	6.3	12.9	8.7	—	2.9
1.2	62.8	3.9	5.6	12.8	8.5	—	2.5
1.3	59.2	4.8	6.4	14.3	10.6	—	2.4
1.1	67.7	4.3	4.5	10.9	9.0	—	2.2
1.6	63.8	4.6	5.1	11.4	10.2	—	2.5

* 16:1 Could not be quantified as the samples were run with an internal standard of 17:0 fatty acid methyl ester. All samples were estimated to have less than 5% 16:1.

Table 11. Distribution of Fatty Acid Residues in Phosphatidylethanolamine in Lung Tissue

14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:4
Control								
0.6	15.7	—	29.7	22.7	7.4	0.8	—	12.6
—	12.1	—	17.0	13.7	3.9	0.6	11.2	29.9
1.0	10.4	1.5	17.3	13.4	3.3	—	9.5	24.9
1.0	12.1	1.3	17.0	13.8	4.2	0.4	4.9	23.4
1.1	16.6	2.2	22.4	19.2	3.3	—	5.7	10.0
97% Oxygen								
0.3	19.2	0.2	36.1	15.4	3.3	—	—	18.2
0.4	10.7	1.3	19.9	15.9	4.5	—	4.4	29.4
0.3	10.8	1.3	15.6	12.7	5.2	—	4.0	30.0
0.4	8.8	1.8	16.9	13.0	5.5	—	7.0	26.7
0.7	13.3	1.5	20.3	17.3	4.7	—	2.6	27.1
1.1	11.4	1.4	16.7	15.1	3.7	—	6.4	22.8

Table 12. Distribution of Fatty Acid Residues in Phosphatidylethanolamine in Alveolar Fluid

14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Control							
2.3	44.0	N.D.	15.4	14.4	6.0.	—	12.9
4.9	41.9	N.D.	26.2	16.6	2.1	—	1.3
4.8	31.6	N.D.	28.0	14.5	2.4	—	2.4
3.4	59.5	N.D.	17.1	8.5	1.5	—	1.2
1.8	36.6	N.D.	14.8	13.6	8.7	—	10.9
1.0	50.1	N.D.	14.5	11.0	7.4	—	10.9
97% Oxygen							
6.3	53.9	N.D.	11.2	7.9	2.3	—	9.3
4.6	47.4	N.D.	13.9	11.7	4.4	—	10.1
2.0	29.9	N.D.	13.4	15.0	5.4	—	14.5
1.8	31.3	N.D.	16.0	18.2	6.9	—	16.2
3.1	39.9	N.D.	9.7	11.4	4.9	—	28.4
3.9	40.3	N.D.	11.9	13.4	5.0	—	14.9

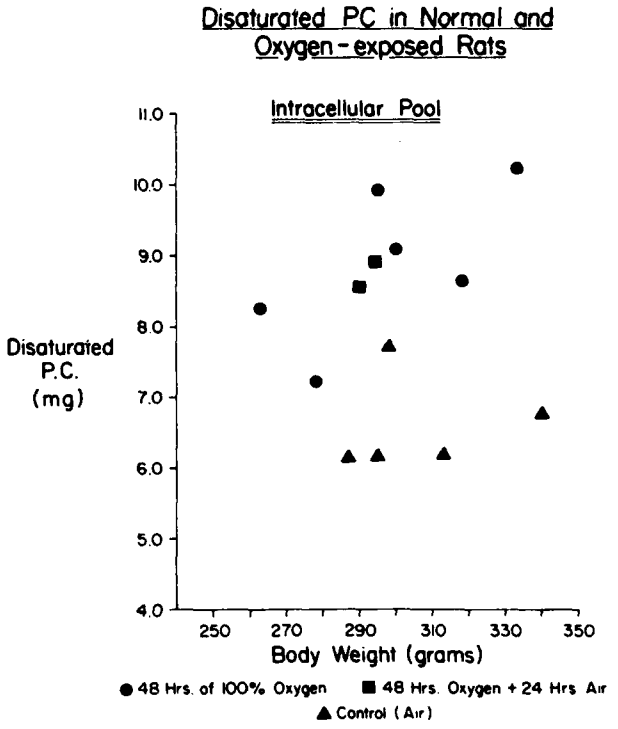

Figure 1. Contents of disaturated phosphatidylcholine (P.C.) found in rat lung before and after breathing 97-100% oxygen at atmospheric pressure.

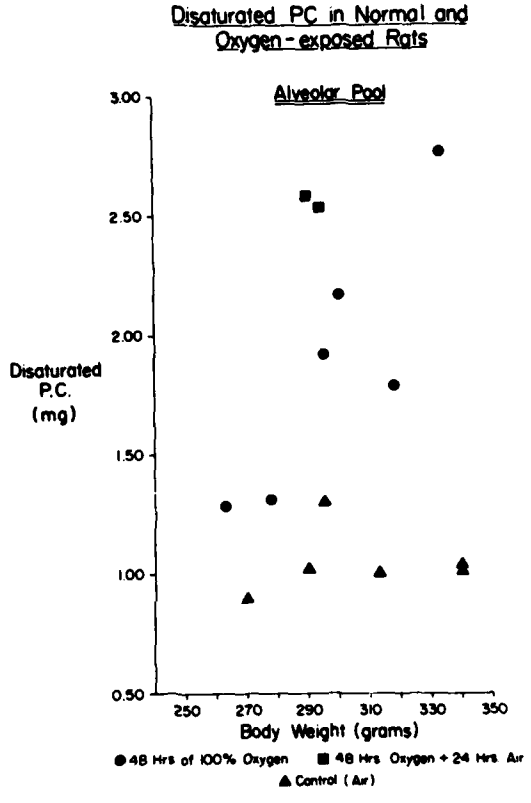

Figure 2. Contents of disaturated phosphatidylcholine (P.C.) found in the alveolar lavage material from rat lungs before and after breathing 97-100% oxygen at atmospheric pressure.

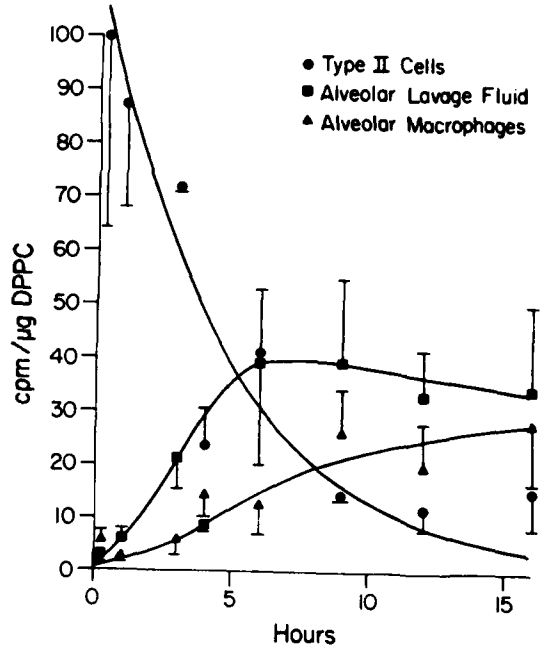

Figure 3. The specific activity of DSPC in Type II cells, alveolar macrophages, and surfactant purified from alveolar lavage fluid. The data are obtained from control (air-breathing) animals. Shown are means and standard errors. A first order exponential curve is fitted to the data obtained from the Type II cells. The others are smooth curves best-fit by eye.

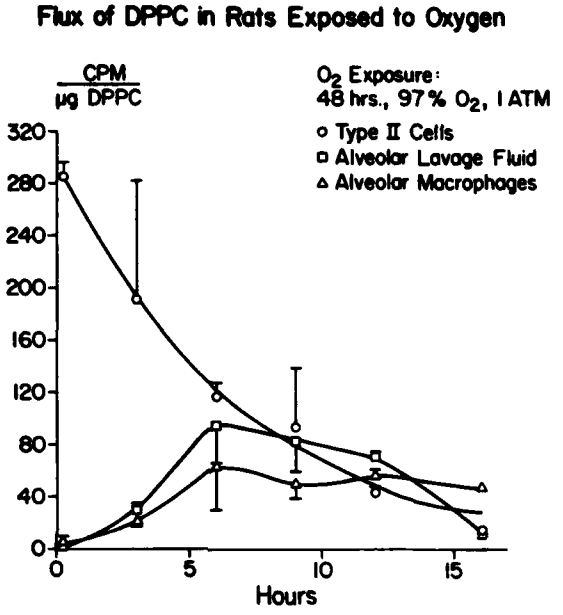

Figure 4. The specific activity of DSPC in Type II cells, alveolar macrophages, and surfactant purified from alveolar fluid. The data are obtained from animals exposed to 97% oxygen for 48 hours. Shown are means and standard errors. A first order exponential curve is fitted to the data obtained from the Type II cells. The others are smooth curves best-fit by eye.

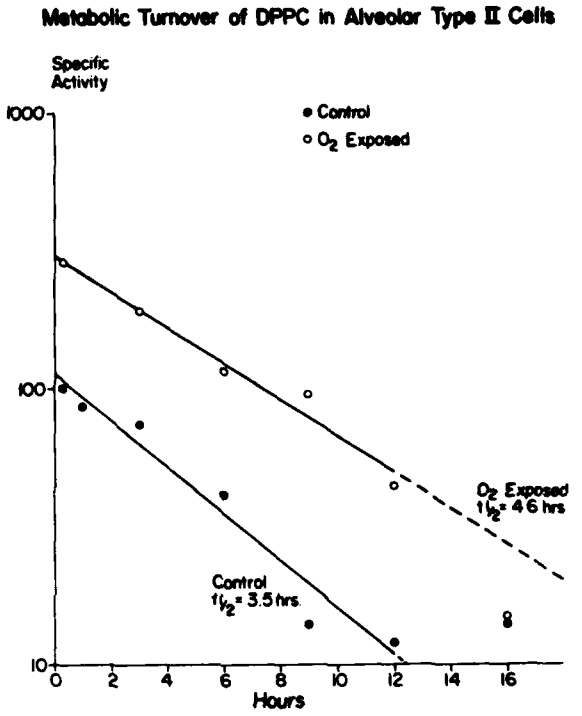

Figure 5. The best fit linear regression curves for the data obtained from Type II cells. The data are those shown in Figures 3 and 4 plotted without standard errors. The slopes of the curves are not different by a test of variance ($P>0.10$).